

AMENDMENTS TO THE SPECIFICATION

Please amend paragraphs [0059]-[0062] on page 13 of the as-filed specification, as follows:

[0059]

After the present yeast suspension was shake-cultured at 30°C for 30 minutes, 150 µl of 70% polyethylene glycol 4000 (Wako Pure Chemical Industries) was added and the mixture was agitated well. Then, after the mixture was shake-cultured at 30°C for 1 additional hour, a heat shock was applied at 42°C for 5 minutes. The ~~bacteria~~ yeast was then rinsed and suspended in 200 µl of water, and this solution was applied to a tryptophan selective culture medium.

[0060]

The obtained colony was applied to a new tryptophan streak culture medium, and selected strains that had demonstrated stability were checked for gene incorporation using PCR analysis. The genome DNA of the yeast to be used for PCR was prepared by shake-culturing a single colony in 2 ml of YPHD media overnight, collecting the ~~bacteria~~ yeast, adding 50 mM Tris-HCL 500 µl and glass beads (425 - 600 µm, acid washed, SIGMA), and putting the mixture through a vortex at 4°C for 15 minutes. The supernatant of this solution was put through ethanol precipitation and was dissolved in 50 µl of sterilized water. Using 5 µl of the prepared genome DNA as the mold, PCR was performed in 50 µl of reaction solution. EX Taq DNA Polymerase (Takara Shuzo) was used for the DNA amplification enzyme, and the PCR amplifier Gene Amp PCR system 9700 (P.E. Applied Biosystems Inc.) was used. The reaction condition for the PCR

amplifier was as follows: the solution was first kept at 96°C for 2 minutes; it was then put through 30 cycles (with each cycle consisting of 96°C for 30 seconds, 55°C for 30 seconds, and 72°C for 90 seconds); and finally was kept at 4°C. The sequences of the primers used were as follows:

LDH-KCB-U : TGG TTG ATG TTA TGG AAG AT (20 mer) (Sequence number 37)

LDH-KCB-D : GAC AAG GTA CAT AAA ACC CAG (21 mer) (Sequence number 38)

PDC1P-U3 : GTA ATA AAC ACA CCC CGC G (19 mer) (Sequence number 39)

[0061]

Those strains that possess a stable tryptophan-synthesizing function and for which PCR was verified under these primers were judged to be transformants into which the LDHKCB gene had been appropriately incorporated. In the present embodiment, three kinds of ~~bacterial~~ yeast strains, namely KCB-27, KCB-210, and KCB-211, were obtained as such transformants. FIG. 9 shows the genome chromosome structures of these yeast *Saccaromyces cerevisiae* transformants.

[0062]

(Embodiment 5: Measurement of L-lactic Acid Production Volume in the Transformants)

Fermentation experiments were conducted on the three kinds of transformants created. As pre-culturing, the transformants were cultured overnight in a YPD solution medium with 2% glucose concentration. After the ~~bacteria~~ yeast were collected and rinsed, they were planted in a YPD solution medium with 15% glucose concentration

such that the ~~bacterial~~ yeast concentration was 1% (0.5 g/50 ml), and were left to ferment at 30°C for several days. This fermented solution was sampled every 24 hours and the volume of L-lactic acid contained was estimated[sic]. For measuring the volume produced, Biosensor BF-4 (Oji Scientific Instruments) was used following the detailed measurement methods described in the user's manual for the instrument.